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Chemical clasto- and anticlastogenicity in mouse micronucleus test

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Abstract. Chemical mutagens ethyl methanesulfonate (EMS) and dimethyl terephthalate (DMtP) were compared in mouse micronucleus (MN) test. Simultaneously, anticlastogenic activity of 1,4-dihydropyridine derivative (DHP) was studied. *CBAxC57Bl/6$_j$* males and pregnant females were exposed to chemicals by i.p. Frequencies of micronucleated polychromatic erythrocytes (MN PCEs) in bone marrow of adults and in fetal liver were analyzed 6, 12, 18, 24, 30, 36, 48 after injections. EMS (300 mg/kg) induced the highest MN PCE frequency in bone marrow 36 h after treatment of adults. The clastogenic effects were the same in both males and females. In fetuses, peak of MN PCEs was observed 24 h after female exposure to EMS, i.e. earlier than in a maternal organism. On contrary, DMtP at the dose of 1/40 LD_{50} induced 4–7,3‰ of MN PCEs in males and was inefficient in pregnant females slightly increasing MN level in fetal cells. Thus, chemicals studied induced different effects depending on physiological status of animals but the both penetrated the placental barrier.
The anticlastogenic effect of DHP (1/10 LD_{50} or 340 mg/kg) was observed at a peak of MN production reaching 30% in males and. 70 % in females, but was inefficient in foetuses. DHP (1/50 and 1/10 LD_{50}) affect MN PCE frequency induced DMtP neither in females nor foetuses. Thus, the antimutagen (AM) studied inhibited EMS clastogenicity in males and females. Anticlastogenic activity was higher in females and was not revealed in foetal cells. This compound was inefficient against DMtP in transplacental test. Data obtained allow the supposition that AM effects are mediated through protective systems of the organism.

Introduction

In the context of increasing environmental mutagenesis and carcinogenesis, the screening and study of new antimutagens have been remaining of great scientific importance and practical interest. Antioxidants are the most promising group of natural and synthetic compounds for this purpose. Some 1,4-dihydropiridine (1,4-DHP) derivatives have been shown to reduce the spontaneous and chemically induced mutation rate in *Drosophila* germ cells [1–2]. The relationship between antimutagenic potential of 1,4-DHP derivatives and their antioxidant capacity as well as a close correlation between efficiency of their action and electron donor activities was established [1–2].

The present investigation was focused on capacity of DHP to inhibit EMS- and DMtP-clastogenicity in mouse micronucleus assays. As known, the MN test allows rather the detection of chromosome breakage [3–4]. Both agents and the antimutagen were administrated by intraperitoneal (i.p.) injections, following that frequencies of micronucleated cells were scored in bone marrow in adults (males and pregnant females)

and in foetal liver. The transplacental MN test permitted identification of events induced by chemicals not only in a maternal organism, but in foetuses too. Besides, we estimated the relationship between two forms of erythrocytes (polychromatic and normochromatic ones) that can indicate a possible toxic effect of the chemicals under study.

As a result of investigations, dynamics of MN formation in mouse bone marrow and foetal liver caused by EMS and DMtP was revealed. Effects of the 1,4-DHP derivative on chemically induced MN production were compared in mouse bone marrow (both in males and pregnant females) and in the foetal liver.

1. Materials and Methods

1.1. Chemicals

Monofunctional alkylating agent ethyl methanesulfonate (EMS, CAS No. 62-50-0 of Sigma production), as well as dimethyl therephthalate (DMtP, CAS No. 12-06-16) was used as a model clastogens. The 1,4-DHP derivative (2,6-dimethyl-3,5-diethoxycarbonyl-4-(Na carboxylate)-1,4-dihydropyridine) was synthesized at the Membrane Active Compound Laboratory of Latvian Institute of Organic Synthesis and were kindly provided for investigation by Prof. G. Duburs.

1.2. Animals and treatment

(C57Bl/6j × CBA) F_1 mice, aged 4–6 weeks, were used for investigations. Males as well as pregnant females of 15 days gestation were treated (i.p.) with an antimutagen and the mutagen step by step. DHP was injected to animals in the doses of 1/10 LD_{50} (340 mg/kg) Mice were exposed to EMS (300 mg/kg) 12 h later. To make necessary concentrations, the chemicals were resolved in distilled water or dimethyl sulfoxide (DMSO) and injected to animals at a volume of 0.2 ml. Mice were received 0.2 ml distilled water in the group of a negative (spontaneous) control and were exposed only to EMS or DMtP in the group of a positive control.

1.3. Bone marrow and transplacental micronucleus assays.

In bone marrow MN test, adult animals were sacrificed by cervical dislocation 6, 12, 18, 24, 36, 48 h after clastogen exposure. Slides of bone marrow were prepared according to Schmid [5] with some modification [6]. In transplacental test, pregnant females were sacrificed 6, 12, 18, 24, 36 and 48 h after clastogen administration. Simultaneously with maternal bone marrow, foetuses were removed and foetal livers were extracted. Cell suspensions and slides were prepared following Cole et al. [7] and Stoyel and Clark [8]. The frequencies of micronucleated polychromatic erythrocytes (MN PCEs), and the ratio of PCEs to total erythrocytes were recorded on the basis of observation no less than of 1000 PCEs and 500 erythrocytes per animal. The results were computer-processed either by χ^2-criterion for comparing MN PCE frequencies or Student's *t*-test for the polychromatic/normochromatic erythrocytes (PCE/NCE) ratio comparison.

2. Results and Discussion

The dynamics of EMS clastogenesis depending on cell sampling time is shown in Fig. 1. In adult mice received the dose of 300 mg/kg, the yield of EMS-induced MNPCEs increased within the interval from 18 to 48 h with a maximum of their induction (21.3‰ in males and 20.4‰ in females) being found 36 h after injection.

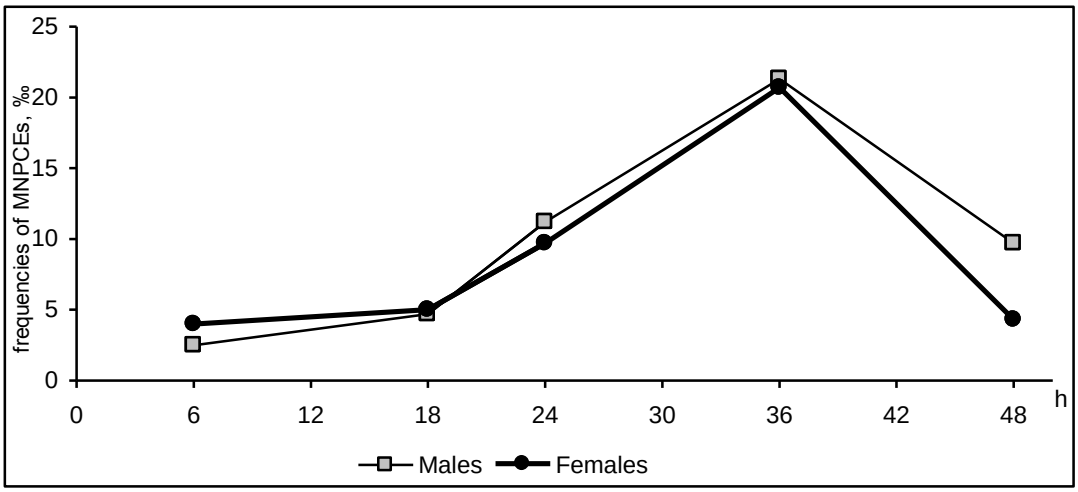


Figure 1. EMS (300mg/kg) induced-MN dynamics
in bone marrow cells of males and females

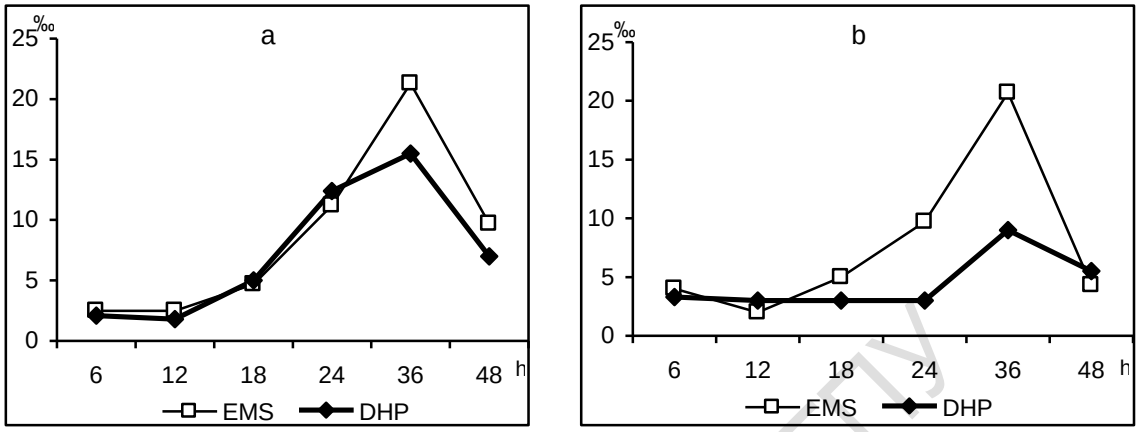
As seen from Table 1, the peak of MN induction in foetuses was observed 24 h later maternal treatment with EMS. In contrast to the alkylating agent, DMtP was inefficient in females, although slightly increased MN PCE frequency in foetuses 48 h after maternal treatment.

Table 1. Clastogenicity of EMS and DMtP in transplacental test

Treatment	Sampling time (h)	Number of		MNPCE frequency (%)	PCE/NCE
		Animals	PCEs		
MNPCE frequencies in pregnant female bone marrow					
Control (negative)		4	4000	0.30	56.98±2.74
EMS 300mg/kg	12	4	4000	0.13	50.13±3.71
	18	6	6000	0.50	43.67±4.62*
	24	6	6000	0.97*	42.48±3.10*
Control (negative)		6	12000	0.09	59.99±1.89
DMtP 1/40LD_{50}	12	4	4000	0.03	60.72±5.38
	18	8	8000	0.09	54.85±2.17
	24	8	8000	0.13	58.38±3.62
	48	8	8000	0.06	55.42±1.31
MNPCE frequencies in foetal liver					
Control (negative)		5	5000	0.28	56.03±1.66
EMS 300mg/kg	12	6	6000	0.47	43.38±2.64*
	18	9	9000	0.57*	49.22±2.07*
	24	6	6000	1.52**	35.26±3.52*
Control (negative)		12	12000	0.11	52.13±1.97
DMtP 1/40LD_{50}	12	8	8000	0.18	65.91±2.59
	18	6	6000	0.17	54.85±2.17
	24	14	14000	0.14	58.02±1.65
	48	12	12000	0.24*	59.53±1.76

It has been shown that DMtP at the dose of 1/40 LD_{50} induces 4–7,3‰ of MN PCEs in males [6]. In present work the same dose seemed to be inefficient being administered to pregnant females. Thus, EMS induced the equal clastogenic effects in males and females, whereas action of DMtP depended on physiological status of animals and the both penetrated the placental barrier and affected chromosome structures in foetuses.

The effects of DHP are presented in Fig. 2. This antimutagen decreased the EMS-induced MNPCE level by 30% in mouse males (Fig. 2a) and by 70% in pregnant females (Fig. 2b). The maximal anticlastogenic effect corresponded to MN induction peak in both cases. Unfortunately, we failed in revealing anticlastogenic effect in respect to EMS-clastogenicity in foetuses.



In transplacental test, DHP was also studied as an anticlastogen against DMtP, but this activity was found nether in females nor in foetuses. The first result was expected against the observed (spontaneous) MN background, but the second one seemed to be due to mechanisms of DHP action.

The data obtained indicate different mechanisms of EMS and DMtP action: the latter is likely to act by mediated ways, e.g. by induction of reactive oxygen species as is known for other phthalates [9]. Involvement of this mechanism explains the differences between effects of this chemical in males and females since all protective mechanisms are the most active during gestation. We think that DHP reduces chemical clastogenicity affecting some protective systems, including detoxifying pathways and/or antioxidant defenses. It makes clearer, why it strongly protects pregnant females as compared to males and especially to foetuses devoid of such protective systems.

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